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SPOTTED FEVER GROUP RICKETTSIAE IN *AMBLYOMMA* TICKS LIKELY TO INFEST HUMANS IN RURAL AREAS FROM NORTHWESTERN ARGENTINA

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Abstract This work was performed to detect *Rickettsia* species of the spotted fever group in *Amblyomma* ticks likely to infest humans in rural areas from northwestern Argentina. Free-living ticks were collected and determined as *Amblyomma tigrinum, Amblyomma neumanni* and *Amblyomma tonelliae*. *Rickettsia* infection was determined by polymerase chain reactions which amplify fragments of the rickettsial genes *gltA* and *ompA*. A high frequency (35/44, 79.5%) of *Candidatus* "Rickettsia andeanae" was observed in *A. tigrinum* ticks, and *Candidatus* "Rickettsia amblyommii" was found in three out of 14 nymphs of *A. neumanni*. All 14 *Amblyomma tonelliae* ticks were negative for rikettsiae. The infection with spotted fever group rickettsiae in ticks aggressive for humans reveals the potential risk of exposure to tick-borne pathogens of people inhabiting rural areas of northwestern Argentina.

Key words: spotted fever rickettsiae, ticks, *Amblyomma,* Argentina

Resumen ***Rickettsias del grupo de las fiebres manchadas en garrapatas del género* Amblyomma*, capaces de infestar humanos, en áreas rurales del noroeste de Argentina.*** El objetivo de este trabajo fue detectar rickettsias del grupo de las fiebres manchadas en garrapatas del género *Amblyomma* capaces de infestar humanos, en áreas rurales del noroeste de Argentina. Se colectaron garrapatas en su fase de vida libre que fueron determinadas como *Amblyomma tigrinum, Amblyomma neumanni* y *Amblyomma tonelliae*. La infección con Rickettsia fue determinada utilizando la reacción en cadena de la polimerasa para amplificar los genes *gltA* y *ompA*. *Candidatus* "Rickettsia andeanae" fue detectada en *A. tigrinum* con alta frecuencia (35/44, 79.5%), mientras que *Candidatus* "Rickettsia amblyommii" fue encontrada en tres de las 14 ninfas de *A. neumanni*. Los 14 especímenes de *A. tonelliae* fueron negativos. La infección de garrapatas capaces de infestar humanos con rickettsias del grupo de las fiebres manchadas evidencia el riesgo potencial al que están expuestos los humanos que habitan o frecuentan áreas rurales del noroeste argentino.

Palabras clave: rickettsias del grupo de las fiebres manchadas, garrapatas, *Amblyomma*, Argentina

Amblyomma (Acari: Ixodidae) is the tick genus with the highest richness of species in South America[1]. This is important for public health since most of the records of ticks biting humans in this continent correspond to *Amblyomma* species[1, 2]. Intracellular bacteria of the genus *Rickettsia* belonging to the spotted fever group are among the most important tick-borne human pathogens in South America, and they are principally vectored by *Amblyomma* species[3]. The taxa *Amblyomma cajennense* sensu lato, *Amblyomma aureolatum, Amblyomma triste, Amblyomma tigrinum* and *Amblyomma ovale* were involved as vectors of human pathogenic rickettsiae[3-5]. In this sense, *Amblyomma* ticks were determined as vectors of *Rickettsia* spp. in most of the human cases of rickettsioses diagnosed in Argentina[3, 5].

A total of 25 tick species of the genus *Amblyomma* were recorded for Argentina, where13 of them were recorded biting humans[6-10]. *Amblyomma* ticks acquire epidemiological relevance in northern Argentina because they are prevalent in areas intended for recreational use (e.g. tourism) and economic activities (e.g. livestock production) where human presence is usual. Therefore, the aim of this work is to analyze *Rickettsia* infection in *Amblyomma* ticks likely to infest humans in rural areas from northwestern Argentina.

Materials and Methods

Free-living ticks were collected in two localities representative of the Yungas Phytogeographic Province (YPP) and Prepuna Phytogeographic Province (PPP)[11] in northwestern Argentina: I) El Carmen (YPP) (24°23′S, 65°15′W), Jujuy Province; II) El Mollar (PPP) (26°57, 65°42´W), Tucumán Province. Questing

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ticks were collected from vegetation by using cloth flags and preserved in 96% ethanol. Species was determined following Estrada-Peña et al.[12] and Nava et al.[10], and also compared with known laboratory-reared specimens deposited in the tick collection of *Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela* (INTA Rafaela), Argentina.

Genomic DNA was obtained by proteolytic digestion with proteinase K, extraction with phenol-chloroform-isoamyl alcohol and precipitation in absolute ethanol as described by Mangold et al.[13]. Initial screening for rickettsiae was carried out with real-time polymerase chain reactions (rt-PCR) assay using primers CS-5 (5_GAGAGAAAATTATATCCAAATGTTGAT -3*)* and CS-6 (5_ AGGGTCTTCGTGCATTTCTT -3), which amplify a 147-bp fragment of the rickettsial citrate synthase (*gltA)* gene[14, 15]. The rt-PCR were performed in a Rotor-Gene-Q6000 (Qiagen). Primers and probe concentrations were optimized in previous assays by spanning different initial concentrations of oligonucleotides. Real-time PCR cycling conditions were as follows: 1 cycle at 95 °C for 2 min, followed by 40 cycles of 15 s at 95 °C, 30 s at 50 °C, and 30 s at 72 °C. This rt-PCR assay has successfully yielded fluorogenic signals for all *Rickettsia* species. For sequencing purposes, rt-PCR-positive specimens were subjected to conventional PCR amplifications targeting fragments of two rickettsial genes, the above-mentioned citrate synthase gene (*glt*A) and the 190-kDa outer membrane protein (*omp*A). In turn, two different primers combinations were used for sequencing this latter gene in different groups of specimens (Table 1)[16-18]. One negative control (water) and two positive controls (DNA of *Rickettsia parkeri* in the analysis of *A. neumanni* and *A. tonelliae*, and DNA of *Rickettsia massiliae* in the analysis of *A. tigrinum*) were included in each PCR run. Products o*f* the conventional PCR amplifications were purified by WIZARD SV PCR purification columns (Promega, Madison, Wis.) according to the manufacturer's protocol, and the purified amplicons were submitted to sequencing.

Sequences were aligned with each other and with the corresponding sequences of the *Rickettsia* species available in GenBank, using the BioEdit Sequence Alignment Editor[19] with the CLUSTAL W program[20]. Pairwise comparison among sequences was performed by using MEGA version 5.0[20]. A phylogenetic analysis was carried out with *ompA* sequences employing the maximum-likelihood method by using the program Mega 5.0[20]. Best fitting substitution model (GTR (G+I)) was determined with the Akaike Information Criterion using the maximum-likelihood model test implemented in MEGA 5.0. Support for the topologies was tested by bootstrapping over 1,000 replications and gaps were excluded from the comparisons.

Results

One male and 14 nymphs of *Amblyomma neumanni* and six males and eight females of *Amblyomma tonelliae* were collected in El Carmen. Forty four adults of *A. tigrinum* (16 males, 28 females) were collected in El Mollar. All ticks were tested for *Rickettsia* species. Thirty five (14 males, 21 females) out of 44 specimens (79.5%) of *A. tigrinum* were found to be *glt*A positive by rt-PCR. DNA sequences were obtained from 21 *glt*A-positive *A. tigrinum* ticks. These sequences were identical among each other (GenBank accession number: KT878724) and they had more than 99.8% similarity with the corresponding sequences of *Candidatus* "R. andeanae" detected in *Amblyomma parvum* from Argentina (GenBank accession number: EF451001) and *Amblyomma maculatum* from Peru (GenBank accession number: GU169050). Three *glt*A-positive samples obtained from *A. tigrinum* were used to amplify a circa 500-bp fragment of *omp*A gene with primers Rr190.70p and Rr190.701R (Table 1). The sequences were identical among each other (GenBank accession number: KT878725) and with those *omp*A sequences of *Candidatus* "R. andeanae" reported from *A. parvum* in Argentina and Brazil (GenBank accession numbers: EF451004, KF030932). Three nymphs of *A. neumanni* were PCR-positive for both *glt*A and *omp*A genes. In these specimens, *omp*A amplicons were obtained with primers Rr190.70p and Rr190.602n (Table1). All three DNA positive samples were sequenced. The similarity of the three *glt*A sequences from El Carmen (GenBank accession number: KT878726) with the corresponding *glt*A sequence of *Candidatus* "R. amblyommii" previously reported in *A. neumanni* ticks from Argentina (GenBank accession number: DQ517290) was 99.7%. In the same way, the three *omp*A sequences obtained from the positive samples of *A. neumanni* (GenBank accession number: KT878727) was 99.5 % similar to the sequence of *Candidatus* "R. amblyommii" also detected in *A. neumanni* ticks from Argentina (GenBank accession

TABLE 1.– *Primer pairs used for amplification and sequencing of rickettsial gene fragments*

Gene	Primers	Nucleotide sequence (5' --- 3')	Product size in basepairs	Reference
gltA	CS-239	GCTCTTCTCATCCTATGGCTATTAT	549	16
	CS-1069	CAGGGTCTTCGTGCATTTCTT		
ompA	Rr190.70p	ATGGCGAATATTTCTCCAAAA	532	17
	Rr190.602n	AGTGCAGCATTCGCTCCCCCT		
	Rr190.70p	ATGGCGAATATTTCTCCAAAA	circa 500	18
	Rr190.701R	GTTCCGTTAATGGCAGCATCT		

number: DQ517292). All 14 *A. tonelliae* ticks were negative for *Rickettsia* species. The phylogenetic position of the *Rickettsia* strains detected in this study according to *omp*A sequences is shown in Fig. 1.

Discussion

The finding of *Candidatus* "R. andeanae" infecting *A. tigrinum* represents the first record of this association for Argentina. This result is not unexpected because *Candidatus* "R. andeanae" was already reported in *A. tigrinum* ticks from Chile[22], and also in other American countries (including Argentina) associated to different species of *Amblyomma* as *A. triste, A. parvum, A.pseudoconcolor* and *A. maculatum*[23-28]. However, the high prevalence detected in this work is noteworthy. Paddock et al.[28] described a high prevalence of *Candidatus* "R. andeanae" in *A. maculatum* from USA (47% in ticks from Kansas, 73% in ticks from Oklahoma), and they suggest that those high levels of *Candidatus* "R. andeanae" infection may be responsible for the exclusion of the human pathogenic *Rickettsia parkeri* from their shared tick host. In this sense, Romer et al.[5] found *A. tigrinum* ticks infected with *R. parkeri* in a tick population from Córdoba Province Argentina, where no ticks were found to be positive to *Candidatus* "R. andeanae". Contrarily, the *A. trigrinum* ticks analyzed during this study were positive to *Candidatus* "R. andeanae" but not to *R. parkeri*. Altogether, these results support the idea of a rickettsial interference between *Candidatus* "R.

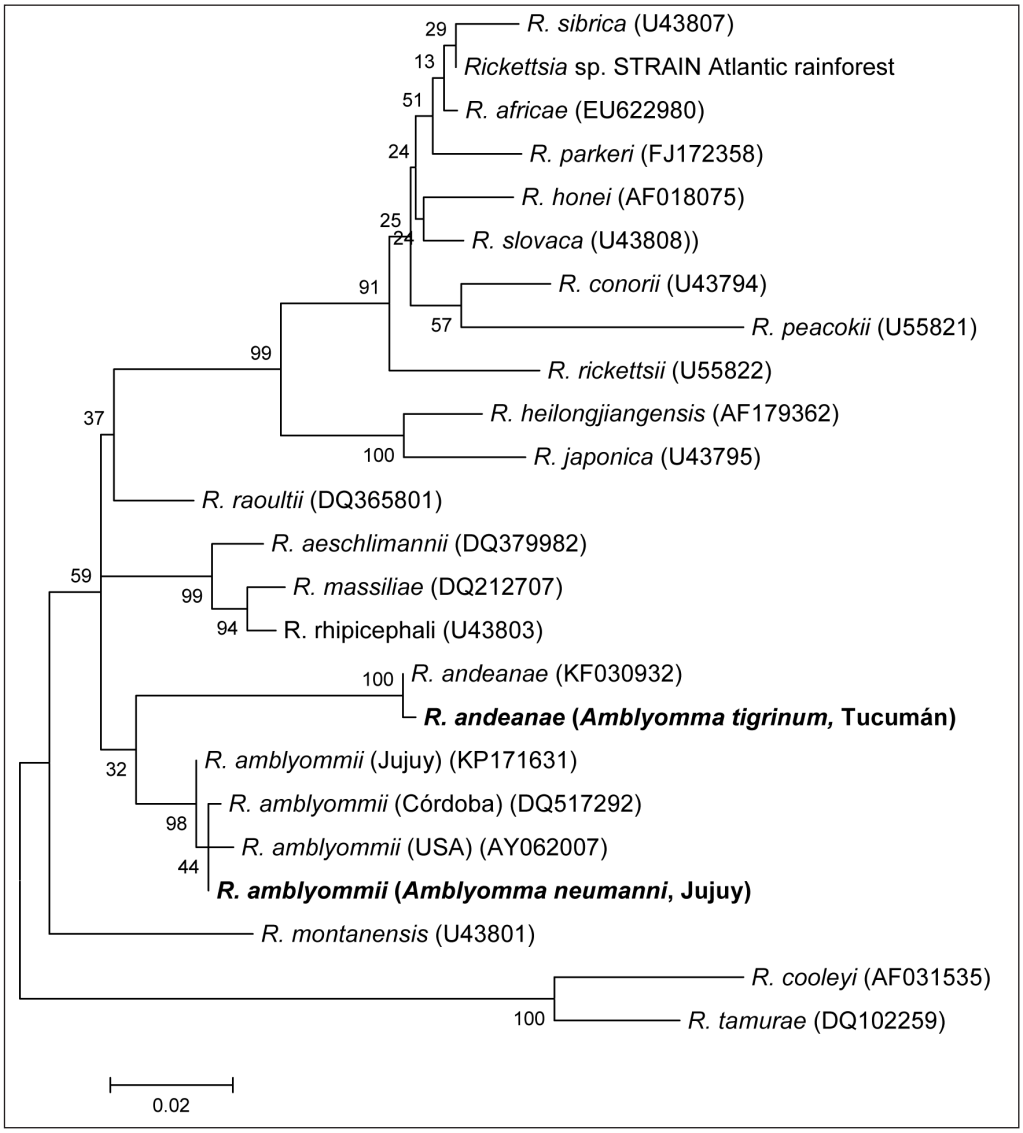

Fig. 1.– Maximum-likelihood tree constructed from ompA partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets.

andeanae" and *R. parkeri*. However, this phenomenon does not appear to be everywhere present, as coinfection of *A. maculatum* ticks with *R. parkeri* and *Candidatus* "R. andeanae" has been described[29].

The prevalence of *Candidatus* "R. andeanae" reported by Ferrari et al.[29] was ≈ 3%, a value much lower than both values reported by Paddock et al.[28] and those found in this work. Probably, the exclusion of *R. parkeri* by *Candidatus* "R. andeanae" may occur only when a tick presents high levels of infection with *Candidatus* "R. andeanae".

A better knowledge of the pathogens carried by *A. neumanni* is relevant in Argentina because this is one of the tick species with the highest numbers of human infections reported in the country[2, 7]. *Candidatus* "R. amblyommii" was previously reported in *A. neumanni* ticks from Córdoba[30] and Salta Provinces (M. Mastropaolo and S. Nava, unpublished observations). With the exception of a sole report of the non-pathogenic *Rickettsia bellii* in Córdoba Province[30], no other tick-borne bacteria has been detected to date in *A. neumanni*.

The results of this work together with data obtained in previous studies indicate that *A. neumanni* infection with *Candidatus* "R. amblyommii" is probably an ubiquitous phenomenon along the distribution of this tick species. All parasitic stages of *A. neumanni* and adult forms of *A. tigrinum* were recorded biting humans in Argentina[2, 7]. We find here these two tick species being infected with spotted fever group rickettsiae in rural areas of northwestern Argentina. Both *Candidatus* "R. andeanae" and *Candidatus* "R. amblyommii" are currently considered of unknown pathogenicity[3]. Phylogenetically, however, they belong to the spotted fever group, which includes the tick-borne pathogenic *Rickettsia* species. In fact, Apperson et al.[31] have suggested that *Candidatus* "R. amblyommii" might have been involved as the pathogenic agent in some human cases of rickettsiosis in USA. We conclude that the infection with spotted fever group rickettsiae in ticks known to be aggressive to humans alerts on a potential risk of exposure to tick-borne pathogens for livestock producers and people participating in outdoor recreational activities in the area represented by the study sites.

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Conflict of interest: None to declare

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Sanatorio Santa Mónica (*). Qué bien han hecho en ponerle ese nombre de mansedumbre al infierno rojo, en el que a todos los semblantes los ha barnizado de amarillo la muerte, y donde entre los cuatro pabellones, dos de hombres, dos de mujeres, sumamos cerca de mil tuberculosos […]. Y el círculo de montañas allá, que superan otras crestas de montes más distantes […]. Y el río que cuando hay sol, destella chapas de luz entre lo verde. […] El médico también está tuberculoso. "Un vértice del izquierdo, nada más". El practicante también, "casi nada, el derecho reblandecido", y así, todos los que nos movemos como espectros en este infierno que lleva un santo nombre, […]. Tomamos mate de la misma bombilla, porque ya no tememos al contagio y bacilo más o menos por "campo" importa poco. Las conversaciones languidecen a poco de iniciadas y, generalmente, guardamos silencio.

Roberto Arlt (1900-1942)

Esther Primavera. En: El jorobadito y otros cuentos. Buenos Aires: Losada, 2006, pp 72, 75, 80
(*): Se trata del *Hospital Colonia Santa María de Punilla, en las inmediaciones de Cosquín, Provincia de Córdoba.*